

## High Oligonucleotide Recovery From Liver Tissue

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This is an Application Brief and does not contain a detailed Experimental section.

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### Abstract

This application brief provides guidance on the extraction of therapeutic oligonucleotides from tissue samples for quantitative analysis and demonstrates high oligonucleotide recovery from Liver Tissue.

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### Experimental

The following (Figure 1) summarizes the solvent-assisted tissue homogenization and oligonucleotide extraction workflow and protocol we use internally with our OligoWorks SPE Microplate Kit. Note we utilize a 1:10 ratio between the mg of tissue being homogenized and the total volume (mL) of homogenization mix, including Rapizyme Proteinase K digestion reagents, buffer and solvent.

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**1** **Bead-based homogenization**  
 1:10 tissue (g)/RapiZyme digestion module reagents  
 100 mg Tissue  
 +30 µL Oligo standard mix  
 +220 µL 100 mM Tris HCl pH 7  
 +300 µL Acetonitrile or methanol  
 +100 µL 0.5 M TCEP HCl  
 +100 µL 6 M GuHCl  
 +250 µL RapiZyme™ proteinase K  
 bead disruption (90s, 6400 rpm) with Precellys 1  
 Tissue Homogenizer  
 1. <https://www.berlin-corp.com/5-tissue-homogenizer>  
 2. BioRender.com.

**2** **RapiZyme™ proteinase K digestion**  
 2 hrs. at 55 °C, 600 rpm



4. <https://www.eppendorf.com/us-en/eShop-Products/Temperature-Control-and-Mixing/Instruments/Eppendorf-ThermoMixer-C-p-PFp19703>

**3** **Sample clarification**  
 Refrigerated microcentrifugation  
 30 min, ≥ 9,400 RCF (10,000 RPM)<sup>2</sup>  
 Process digested/homogenized tissue supernatant



3. <https://www.eppendorf.com/us-en/eShop-Products/Centrifugation/Microcentrifuges>

**4** **OligoWorks SPE (WAX) SPE Microplate**  
**Sample loading:** 200 µL of a 1:1 homogenized/digested tissue supernatant (100 µL) diluted with 50 mM NH<sub>4</sub>OAc pH 5.5 Buffer (100 µL)  
**Wash 1:** 200 µL 50 mM NH<sub>4</sub>OAc pH 5.5 Buffer  
**Wash 2:** 200 µL 30% Methanol  
**Elute:** 2 × 25 µL OligoWorks SPE Eluent  
**Dilution:** 1:1 Water Diluent for Eluate (Optional)  
**LC-MS analysis:** Up to 30 µL direct injection to DIPEA IP-RPLC




Figure 1. Oligonucleotide tissue extraction workflow and protocol using solvent assisted tissue homogenization and digestion with RapiZyme Proteinase K Digestion Module and OligoWorks SPE Microplate-2 mg/well.

Note: equipment referenced is what we use in our lab, but alternate equipment with equivalent capabilities may be used instead.

## LC-MS Analysis

### LC-MS ANALYSIS

|                   |   |
|-------------------|---|
| UPLC              | ACQUITY™ Premier BSM System FTN   |
| MPA               | 1% HFIP (hexafluoro-2-propanol) 0.1% DIPEA (N,N-Diisopropylethylamine) H <sub>2</sub> O (Water) |
| MPB               | 0.75% HFIP 0.0375% DIPEA, 65% Acetonitrile (ACN)  |
| Column sorbents   | ACQUITY Premier Oligonucleotide C <sub>8</sub> Column, 130Å, 1.7 µm 2.1 x 50 mm                 |
| Col temp.         | 50 °C   |
| Sample temp.      | 10 °C   |
| Inj. volume       | 5-15 µL   |
| Purge solvent     | 90:10 H <sub>2</sub> O:MeOH (Methanol)  |
| Wash solvent      | 25:25:25:25 Water:MeOH:ACN:IPA  |
| MS                | Xevo™ TQ-Absolute   |
| Capillary (kV)    | 2.0   |
| Desolvation temp. | 500 °C  |
| Desolvation flow  | 1000 L/Hr   |
| Cone gas flow     | 150 L/Hr  |

| Time (min) | Flow (mL/min) | %A | %B | Curve |
|------------|---------------|----|----|-------|
| 0.00       | 0.6           | 95 | 5  | 6     |
| 3.25       | 0.6           | 77 | 23 | 6     |
| 3.75       | 0.6           | 10 | 90 | 6     |
| 4.1        | 0.6           | 10 | 90 | 6     |
| 4.25       | 0.6           | 95 | 5  | 6     |



## Results and Discussion

### High oligonucleotide tissue recovery using solvent assisted tissue homogenization and digestion with OligoWorks™ SPE Microplate Kit

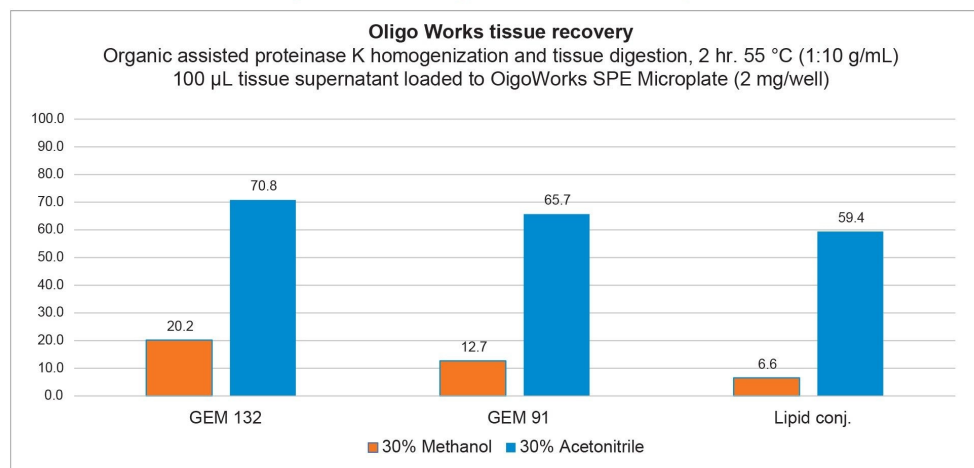


Figure 2. Demonstration of OligoWorks SPE Microplate performance, with >70% oligonucleotide recovery using 0.05 g tissue/0.5 mL Proteinase K Digestion Module reagents (homogenized and digested 2 hrs at 55 °C, 600 rpm), and 100 µL of tissue supernatant purified using the OligoWorks SPE Microplate Kit, containing OligoWorks RapiZyme Proteinase K Digest Module and OligoWorks SPE Microplate-2 mg/well.

## Ordering Information

| Description  | P/N       |
|--|-----------|
| OligoWorks SPE Microplate Kit  | 186010614 |
| ACQUITY Premier Oligonucleotide C <sub>18</sub> Column, 130Å, 1.7 µm 2.1 × 50 mm | 186009484 |
| QuanRecovery™ with MaxPeak, 700 µL plate   | 186009184 |
| Polypropylene cap mat round well for 96-well                                     | 186009452 |

## Featured Products

[ACQUITY Premier System <https://www.waters.com/waters/nav.htm?cid=135077739>](https://www.waters.com/waters/nav.htm?cid=135077739)

[Xevo TQ Absolute Triple Quadrupole Mass Spectrometer <https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/xevo-tq-absolute.html>](https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/xevo-tq-absolute.html)

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